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Background

Necrotising fasciitis (NF), commonly known as flesh eating disease is a fast progressing, potentially lethal infection of the subcutaneous tissue/fascia. Because of the speed of infection immediate diagnosis and therapy (including systemic antimicrobials and aggressive surgical debridements) are required.

The infection is often caused by streptococci (especially *Streptococcus pyogenes), Staphylococcus aureus* and *Clostridum sp.*[1]

Objective

Accurate identification of the microorganisms may add to the knowledge of NF pathogenesis and influence the administration of antibiotics, and thereby potentially improve the outcome for the patients. Here we investigate the applicability of different molecular methods compared to standard culture-based methods.

Methods

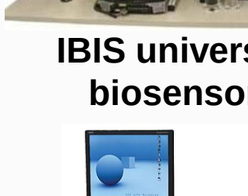
Culture

DNA extraction for molecular methods:

IBIS universal biosensor

Roche 454 pyrosequencing

Clone libraries of near full-length 16S rRNA



Quantitative PCR

TABLE 1: Overview of samples included in this study. All samples were obtained during tissue debridement at Rigshospitalet (Copenhagen, Denmark).

Patient	No.	Sample site
1	1	Arm
	2	Arm
	3	Arm
	4	Arm
	5	Arm
2	6	Shoulder
	7	Shoulder
	8	Shoulder
3	9	Arm
	10	Arm
4	11	Vulva
5	12	Neck
	13	Neck
6	14	Shoulder
	15	Shoulder
7	16	Crus
	17	Crus
8	18	Femur
	19	Femur
	20	Femur
	21	Femur

•Samples 1-5 (Patient 1) were used to evaluate DNA extraction method.

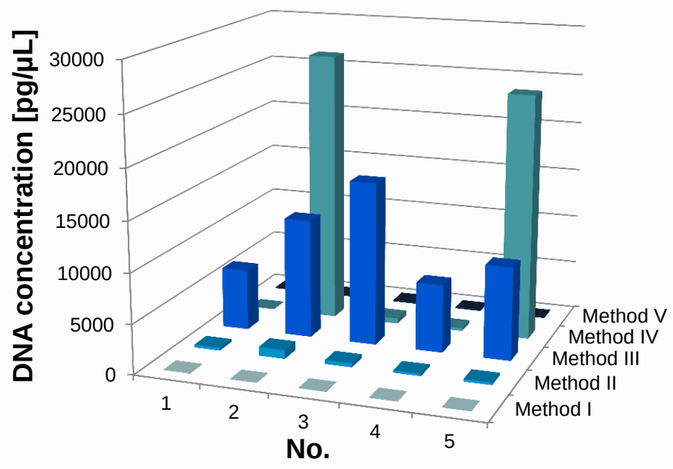
DNA extraction test

•DNA was extracted from sample no. 1-5 using methods I-V (Figure 1).

FIGURE 1: Schematic overview of the tested DNA extraction methods. Protocol A: DNeasy Blood & Tissue kit (Quiagen), B: Proteinase K + FastDNA Spin kit (MP Biomedicals) and C: MolYsis Complete5 (MolZym).

•Method III and IV gave the highest DNA yield since no MolYsis (Molzym) pretreatment was used (Figure 2).

FIGURE 2: Quantity of DNA determined using Picogreen dsDNA dye (Invitrogen).



•Clone libraries were constructed for DNA extracted by all methods from sample no. 3.

TABLE 2: Most frequently occurring bacteria in clone libraries for sample no. 3 using extraction methods I-V. An x indicates that the bacteria was found in the clone library for the respective extraction method.

	Method				
	I	II	III	IV	V
Streptococcus sp.	x	x	x	x	x
Pseudomonas aeruginosa		x	x		
Escherichia coli		x	x	x	
Ralstonia pickettii		x	x		x
Klebsiella sp.			x		
Propionibacterium acnes			x		

•DNA extraction method III was chosen for the remaining samples.
•The protocol was extended to include a bead-beating step.

Results - quantity

•Quantitative PCR confirmed findings of *S. pyogenes* by culture and molecular methods.
•*S. pyogenes* was quantified in sample no. 13 although the bacteria was not found previously.
•Samples where *S. pyogenes* was not dominant corresponded to samples where other microorganisms had been identified (no. 9, 11 and 13).

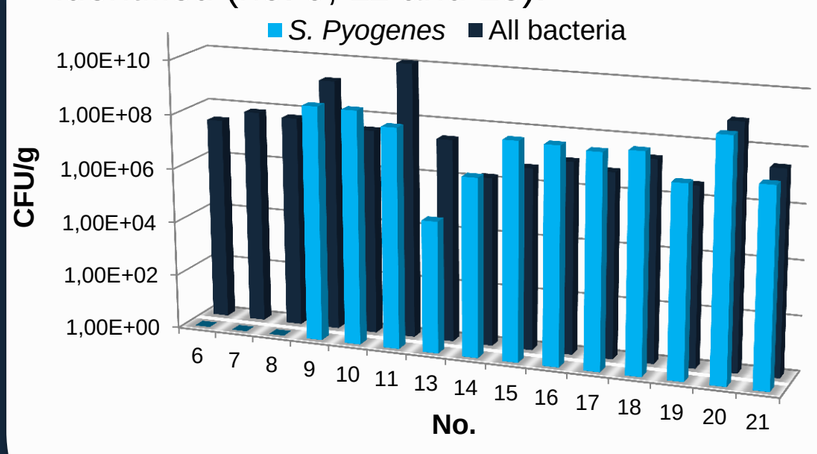


FIGURE 3: Quantitative measurements of *S. pyogenes*[2] and the 16S rRNA gene of all bacteria[3]. Gene copies were converted to a measure of CFU per gram tissue sample.

Results - Identity

•Generally the methods used for identification of microorganisms gave concordant results (Table 3).
•In some cases culture resulted in no growth of microorganisms, although bacteria could be found by molecular methods.
•Different molecular methods gave concordant results for the most frequent bacteria.

TABLE 3: Identity of microorganisms determined by culture compared to microorganisms determined by molecular methods (A: IBIS, B: 454 pyrosequencing and C: clone library).

No.	Culture	Molecular methods
6	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*[A, B]
7	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*[A, B] *Staphylococcus cohnii*[A] *Thauera terpenica*[A]
8	No growth	*Streptococcus pneumoniae*[A, B] *Staphylococcus epidermidis*[A] *Staphylococcus hominis*[A]
9	*Streptococcus* sp.	*S. pyogenes*[A, B] *Streptococcus didelphis*[A, B] *Staphylococcus epidermidis*[A]
10	No growth	*S. pyogenes*[C]
11	*Escherichia coli* *S. pyogenes*	*Escherichia coli*[A, B] *S. pyogenes*[A, B] *Bacteroides fragilis*[A, B] *Staphylococcus hominis*[A] *Staphylococcus epidermidis*[A] *Staphylococcus warneri*[A] + mecA gene[A] *Cladosporium cladosporioides*[A] *Porphyromonas* sp.[B] *Mycoplasma* sp.[B]
12	Fungus	*Candida albicans*[A] *Mycoplasma* sp.[A, B] *Fusobacterium necrophorum*[A, B] *Staphylococcus auricularis*[A] *Staphylococcus saprophyticus*[A] *Prevotella* sp.[B]
13	Fungus	*Mycoplasma salivarium*[C] *Fusobacterium necrophorum*[C] *Mogibacterium* sp.[C]
14	No growth	*S. pyogenes*[C]
15	No growth	*S. pyogenes*[C]
16	*S. pyogenes*	*S. pyogenes*[C] Uncultured bacterium[C]
17	*S. pyogenes*	*S. pyogenes*[C]
18	*S. pyogenes*	*S. pyogenes*[C]
19	*S. pyogenes*	*S. pyogenes*[C]
20	*S. pyogenes*	*S. pyogenes*[C]
21	*S. pyogenes*	*S. pyogenes*[C]

Conclusion

•The bacteria most often found in the samples was *S. pyogenes*.
•Interestingly, one patient was found to harbour no Streptococci but *Candida albicans*, *Mycoplasma* sp. and *Fusobacterium necrophorum*.
•The molecular methods gave concordant results, and confirmed positive culture findings. However, additional microorganisms were identified.
•Advantages of molecular methods: 1) identification of the pathogen(s) after administration of antibiotics and 2) less time-consuming than conventional culture.

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